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Genetics

Localization of the human insulin gene to the distal end of the short arm of chromosome 11

(gene mapping/*in situ* hybridization/recombinant DNA)

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ABSTRACT **The human insulin gene was mapped by hybridization *in situ* of ^{3}H-labeled recombinant plasmid pHIG900 to chromosome preparations in the presence of 10% dextran sulfate. pHIG900 contains a 900-base pair insert of human genomic DNA that includes the coding region for the C peptide and intervening sequence 2. A significant percentage of hybridized cells (34%) exhibited silver grains on the distal end of the short arm (band p15) of chromosome 11. Furthermore, silver grains on this region represented 26% of all chromosomal label. These results demonstrate chromosomal localization of the human insulin gene to 11p15.**

Insulin, a polypeptide hormone produced by beta cells of the pancreas, is required for normal glucose homeostasis. Insulin deficiency results in diabetes mellitus, in which the consequent impairment of glucose utilization results in a profound imbalance in lipid and protein production and metabolism. Although the main clinical symptoms can be alleviated by administration of the hormone, diabetes and its complications are now the third leading cause of death in the United States.

Insulin consists of two polypeptide chains (A and B), which are linked by disulfide bonds. It is synthesized as a single polypeptide chain called preproinsulin (1, 2), which is then processed to form the mature two-chain structure of the hormone. This involves removal of the amino-terminal extension involved in secretion from the pancreatic beta cells to form proinsulin, followed by selective removal of an internal portion of the molecule (connecting or C peptide) (3).

In humans, several lines of evidence suggest the presence of one insulin gene per haploid complement. These include the finding of a single insulin species (4), analysis of genetic disorders in which mutant insulin alleles are inherited in an autosomal dominant pattern (5, 6), and sequence analysis of cloned insulin-coding DNA (7, 8). This is in contrast to other animals, such as rat, mouse, and several fish species, which have two insulin genes (9).

Recent gene mapping studies assigned the human insulin gene to chromosome 11. This was accomplished by probing a panel of human–mouse somatic cell hybrids containing different combinations of human chromosomes with cloned rat and human preproinsulin cDNAs (10). In the present work, ^{3}H-labeled recombinant plasmid pHIG900 was hybridized *in situ* to human chromosome preparations. pHIG900 contains a 900-base pair (bp) genomic insert consisting of most of the C peptide coding region plus the intervening sequence that interrupts this region (IVS 2). Hybridization reactions were in the presence of 10% dextran sulfate which, along with the use of cloned probes, increases the amount of signal and allows the detection of single-copy sequences (11). Highly significant labeling of the distal end of the short arm of chromosome 11 was observed, indicating that the human insulin gene is located on band 11p15.

MATERIALS AND METHODS

Preparation of Plasmid DNA. Recombinant plasmid pHIG900 was constructed by insertion of a 900-bp *Pst* I genomic DNA fragment into the *Pst* I site of pBR322. This fragment was obtained from a recombinant λCh4A phage that contains the entire insulin gene (7). Growth of the plasmid in *Escherichia coli* K-12 RR1 and isolation of plasmid DNA were carried out as described by Katz *et al.* (12). The approved EK-1 host–vector system was used with P1 physical containment in accordance with the National Institutes of Health guidelines for recombinant DNA research.

Radiolabeling of Probe. pHIG900 DNA was ^{3}H-labeled by nick-translation using [^{3}H]dCTP (60.4 Ci/mmol), [^{3}H]dATP (41.2 Ci/mmol), and [^{3}H]dTTP (78.1 Ci/mmol) (New England Nuclear; 1 Ci = 3.7×10^{10} becquerels) according to Lai *et al.* (13). The DNA, labeled to a specific activity of 2×10^7 cpm/μg, was separated from free [^{3}H]dNTPs by centrifugation through 1 ml of hydrated Sephadex G-100 at 1800 × *g*.

Preparation of Human Mitotic Chromosome Spreads. Mitotic chromosome preparations were obtained from human peripheral blood lymphocyte cultures. After incubation for 72 hr, growing cells were synchronized with 0.1 μM amethopterin and then harvested (14). Chromosome preparations were used within 15 days for *in situ* hybridization.

***In Situ* Hybridization.** Hybridization was carried out essentially as described by Harper and Saunders (11). Chromosome preparations were treated with pancreatic ribonuclease A (Sigma) at 100 μg/ml in 2× NaCl/Cit at 37°C for 1 hr, rinsed well in 2× NaCl/Cit, and dehydrated in ethanol (1× NaCl/Cit is 0.15 M NaCl/0.015 M trisodium citrate, pH 7.0). The chromosomal DNA was denatured by immersion of slides in 70% (vol/vol) formamide/2× NaCl/Cit, pH 7.0, at 70°C for 2 min, followed by dehydration in ethanol. ^{3}H-Labeled pHIG900 DNA, at a concentration of 0.05–0.2 μg/ml in 50% (vol/vol) formamide/2× SCP (0.3 M NaCl/0.03 M trisodium citrate/0.04 M $NaPO_4$, pH 6.0)/10% dextran sulfate (Pharmacia), pH 7.0, and with 1000-fold excess sonicated salmon sperm DNA as carrier, was hybridized to the chromosome preparations for 11 hr at 37°C. Slides were rinsed well in 50% formamide/2× NaCl/Cit, pH 7.0, at 39°C and then in 2× NaCl/Cit, followed by dehydration in ethanol. Preparations were exposed to Kodak NTB2 nuclear track emulsion (Eastman) for 5–11 days at −80°C, then developed in Kodak Dektol for 2 min, fixed, and thoroughly rinsed with tap water. The hybridized chromosomes

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Abbreviations: bp, base pair(s); kb, kilobase pair(s); NaCl/Cit, 0.15 M NaCl/0.015 M trisodium citrate, pH 7.0.

were G-banded by staining and then destaining-restaining several times with Wright's stain (15).

RESULTS

In situ hybridization of the ^{3}H-labeled recombinant plasmid pHIG900 to human chromosomes was carried out at two probe concentrations. Cells hybridized at 0.2 μg/ml for 11 hr and exposed to autoradiographic emulsion for 11 days exhibited an average of 1.5 silver grains per cell and low background. A representative chromosome spread from these experiments that exhibits a grain on the distal portion of the short arm of chromosome 11 (arrow) is shown in Fig. 1. The distribution of labeled sites in 35 metaphase cells from this hybridization is shown in Fig. 2, in which each dot represents the position of one silver grain. Twelve (34%) of the cells exhibit label on the distal portion of the short arm of chromosome 11 (band p15). Of 51 total chromosomal sites labeled in the 35 cells, 13 (26%) are located on this region of chromosome 11. Furthermore, of all grains observed on chromosome 11, 80% (12/15) are located on band p15.

Analysis of preparations hybridized at the same concentration (0.2 μg/ml) but exposed for only 5 days showed a very low frequency of labeling (<1 grain per cell). However, of 19 labeled chromosomes 11 analyzed, 70% (14/20) of the grains were located on band p15 of the short arm. Similar results were obtained in experiments in which the probe was hybridized at a concentration of 0.05 μg/ml and slides were exposed for 11 days. Although overall labeling was low, analysis of the chromosomes 11 indicated that 67% of all grains observed on this chromosome were located on band p15.

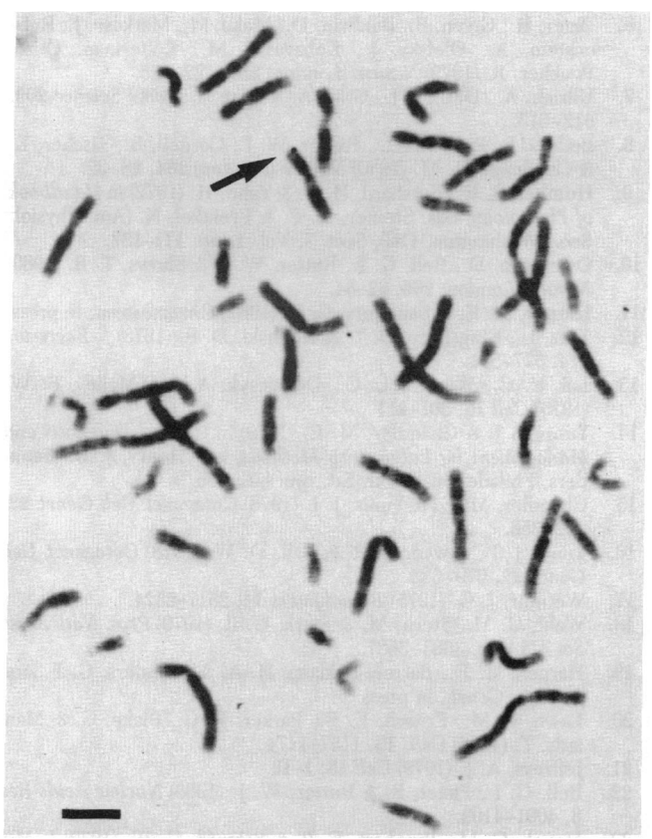

FIG. 1. Human metaphase cell hybridized with ^{3}H-labeled pHIG900 at a concentration of 0.2 μg/ml for 11 hr and exposed to autoradiographic emulsion for 11 days, illustrating typical labeling of band p15 on the distal end of the short arm of chromosome 11 (arrow). Bar, 5 μm.

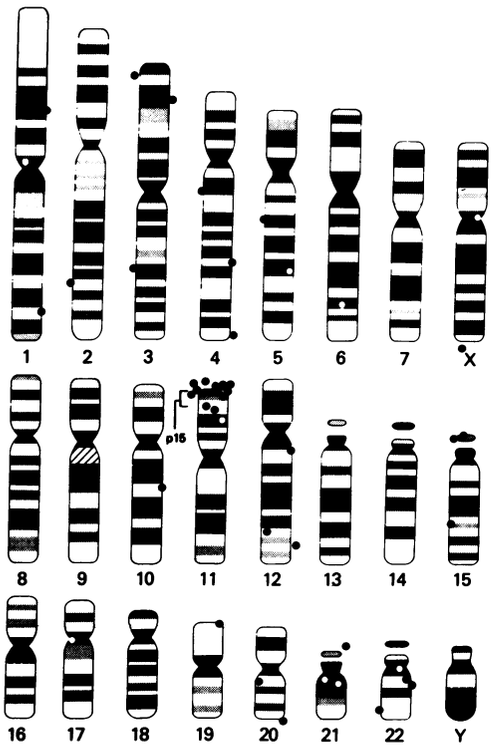

FIG. 2. Composite from 35 cells hybridized *in situ* with ^{3}H-labeled pHIG900 at a concentration of 0.2 μg/ml for 11 hr and exposed for 11 days. Of these cells, 12 (34%) exhibited label (dots) on band 11p15, which represent 26% (13/51) of total labeled sites throughout the chromosome complement. Chromosome idiogram from Yunis *et al.* (16).

A compilation of grain positions from 29 labeled chromosomes 11 from cells hybridized at 0.2 μg/ml and exposed for either 5 or 11 days is shown in Fig. 3. It can be seen that the major site of hybridization with the insulin-specific probe is band p15 on the short arm, allowing assignment of the human insulin gene to this small chromosome segment.

DISCUSSION

The human insulin gene was mapped to a specific chromosome band (11p15) by hybridization of a radioactive insulin-specific sequence to human chromosome preparations. Detection of the ^{3}H-labeled probe was made possible by both the addition of 10% dextran sulfate to the hybridization reaction mixture and the use of cloned probes. Dextran sulfate accelerates the hybridization rate of DNA, promoting the formation of DNA networks between single-stranded, randomly cleaved molecules (17, 18). When a cloned sequence is used, the attached vector molecules cannot hybridize to the chromosomal DNA, but they are free to participate in network formation, increasing the amount of radioactivity deposited at the specific chromosome site. This procedure not only allows direct gene mapping of sequences present in very few copies per haploid genome but also is relatively rapid. With this method, a number of cloned sequences have been localized on human chromosomes, including a 15-kilobase pair (kb) single-copy sequence to band 1p36 (11) and a 550-bp placental lactogen sequence to 17q22-24 (19).

In the experiments described here, 34% of cells exhibited label on band 11p15. Increased time of autoradiographic exposure results in a concomitant increase in both specific and nonspecific labeling. However, because there are a limited number of specific sites available, the signal-to-noise ratio decreases with long exposure times.

Evidence showing that the insulin gene is indeed present in

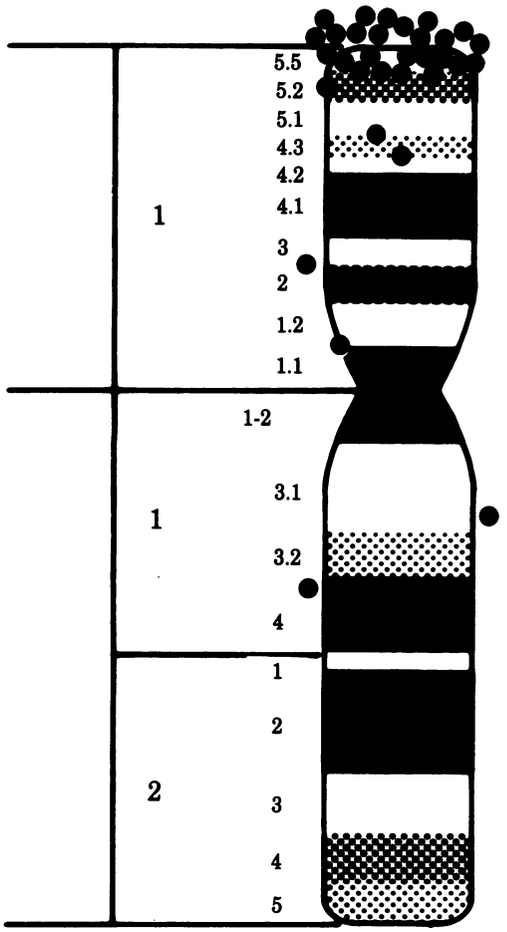

FIG. 3. Distribution of silver grains on 29 labeled chromosomes 11 from cells hybridized with ^{3}H-labeled pHIG900. Of the 34 grains observed, 27 (79%) are located on the distal end of the short arm (band p15), localizing the human insulin gene to this chromosomal segment.

only one copy per human haploid genome has been obtained from protein analysis, genetic studies, and more recently from analysis of the insulin-coding regions of genomic DNA (7, 8). Restriction enzyme mapping in conjunction with Southern blot hybridizations revealed two types of gene sequences. They exhibit single-nucleotide differences in only four positions, which are located in either the intervening sequences or the 3′ untranslated region of the gene. On the basis of frequencies of nucleotide changes in other genes (12, 20, 21), the observed differences between the two types of sequences are believed to reflect allelic variation (7, 8).

Use of a genomic clone containing all of one intervening sequence (IVS 2) as well as C-peptide-coding DNA was possible due to the finding that this fragment is a single-copy sequence. In fact, of the 19 kb of genomic DNA analyzed that contains the 1.4-kb insulin gene, no repetitive sequences are present within 5.7 kb on the 5′ side and 11.5 kb on the 3′ side except for 500 bp located 6 kb from the 3′ end of the gene (22). This 500-bp sequence contains a member of the *Alu* repeat family (23) as well as another less highly repeated homopolymeric segment. It is also interesting that no pseudoinsulin genes were detected within the 19 kb of DNA analyzed, as shown by lack of sequence homology between subregions around the insulin gene (22). Because only one *Eco*RI band is detected on a Southern blot of total human DNA when it is hybridized with insulin cDNA (8), it was not surprising to find that only a single site in the human chromosome complement formed stable hybrids with the insulin gene probe.

Analysis of hybridized cells showed a highly significant labeling of band p15 on the short arm of chromosome 11. In fact, the observation that a large portion of these grains are actually centered on subband p15.3 or located off the distal end of the short arm (Fig. 3) suggests that the insulin gene may actually be located within subband 11p15.3. On the basis of percentage of the total chromosome complement length, this subband most likely contains several thousand kb of DNA.

Refined localization of the insulin gene should prove valuable in the diagnosis and treatment of diabetes. Although the etiology of this disease is poorly understood due to the involvement of environmental and genetic factors (24, 25), some forms result from an altered insulin protein and are likely due to alterations in the insulin gene (5, 6). Knowledge of the gene map surrounding the insulin gene may allow the use of DNA restriction fragment length polymorphisms in detecting individuals with genetic disorders in the insulin gene.

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